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14. ABSTRACT

The incidence of renal cell carcinomas (RCCs) has been steadily increasing, in part due to widespread use of cross-sectional imaging. RCCs have a wide range of aggressiveness, which is currently difficult to assess noninvasively. This has resulted in overtreatment of indolent tumors, and possibly under-treatment of aggressive ones. Therefore, there is an unmet clinical need to be able to reliably distinguish renal cancer aggressiveness for optimal triage of therapies. Hyperpolarized (HP) 13C magnetic resonance spectroscopic imaging (MRSI) is a new metabolic imaging approach that is capable of interrogating specific enzymatic pathways in real time. Building on our previous work, on immoratalized cells, we aim to interrogate tumor metabolism in more clinically relevant RCC tumor models by employing patient-derived tumor tissues in conjunction with HP MRSI in this work. The patient-derived tumor models, including tissue slices maintained in a bioreactor and orthotopic mouse model, can better recapitulate the heterogeneous range of RCCs and facilitate identification of clinically relevant markers of tumor aggressiveness. During the first year, we have successfully established the *in vivo* animal model even with low grade tumors and have accomplished bioreactor *HP* experiments using live patient derived tumor tissue.

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Nothing Listed

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1. INTRODUCTION:

The incidence of renal cell carcinomas (RCCs) has been steadily increasing, in part due to widespread use of cross-sectional imaging [1]. RCCs have a wide range of aggressiveness, which is currently difficult to assess noninvasively. This has resulted in overtreatment of indolent cancers, and possibly under-treatment of aggressive ones. Additionally, current imaging techniques cannot distinguish benign renal tumors from RCCs, resulting in unnecessary surgical resection of benign renal tumors. Therefore, there is an unmet clinical need to be able to reliably distinguish renal tumor aggressiveness for optimal triage of therapies. Hyperpolarized (HP) ^{13}C magnetic resonance spectroscopic imaging (MRSI) is a new metabolic imaging approach that is capable of interrogating specific enzymatic pathways in real time. In this proposal, we aim to interrogate tumor metabolism in a clinically relevant renal tumor models by employing patient-derived tumor tissues in conjunction with HP MRSI. The patient-derived tumor models, including tissue slices maintained in a bioreactor and implanted orthotopically in mice, can better recapitulate the heterogeneous range of renal tumors (including benign renal tumors and RCCs), and facilitate identification of clinically relevant markers of tumor aggressiveness. Specifically,

Aim 1:Identify HP ^{13}C metabolic markers that discriminate benign renal tumors from RCCs, and low grade from high grade RCCs using human TSCs cultured in a bioreactor.

Aim 2:Identify HP ^{13}C metabolic markers that discriminate *low* grade from high grade RCCs using human tumor tissues implanted under renal capsule of mice.

There has been no change in the tasks specified in Aims 1 and 2 from those proposed in the original Statement of Work, and below I describe the research that has been accomplished for each aim.

2. KEY WORDS:

Renal Cell Carcinoma, Hyperpolarized 13C MR, Sub-renal capsule, patient derived tissue slice cultures, bioreactor

3. OVERALL PROJECT SUMMARY:

Aim 1: Ex vivo bioreactor experiments

Summary: The key finding from the bioreactor experiments using patient-derived renal tumor tissues is that clear cell RCCs (the most common and usually more aggressive type of RCCs) have rapid lactate efflux into extracellular space when compared to benign renal tumors and normal renal tissues. Because extracellular lactate and acidic extracellular pH are implicated in tumor aggressiveness and metastatic potential, our findings suggest that lactate efflux, measured with hyperpolarized MR in conjunction with diffusion weighted MRI currently being developed to quantify the relative amount of extracellular lactate, can serve as a potential biomarker to stratify renal tumor aggressiveness.

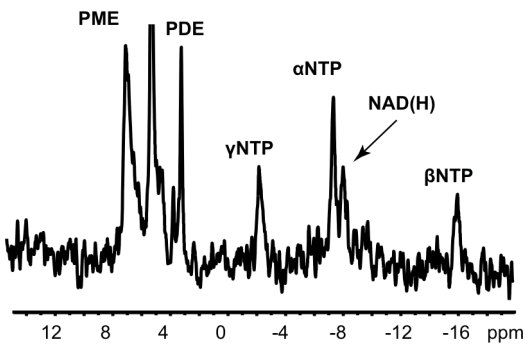


Figure 1. ^{31}P spectra from 4 slices of patient derived RCC TSC in the bioreactor (~60 mg).

Thus far we have successfully acquired hyperpolarized metabolic measurements on 3 benign tumors (2 oncocytomas and 1 angiomyolipoma), 9 clear cell RCC's (ccRCC), and 12 normal renal tissues. 31P NMR of the tissue slices in the bioreactor showed the maintenance of bNTP (Fig 1), indicating viability of the tissues during the hyperpolarized studies. The bNTP peak was also used to normalize the hyperpolarized signals across experiments.

We then recorded the dynamic hyperpolarized 13C pyruvate metabolism of the tissue slices over 5 minutes. **Figure 2** shows representative spectra summed over the dynamic time course in ccRCC tissue slices. **Figure 3** summarizes the observed hyperpolarized alanine

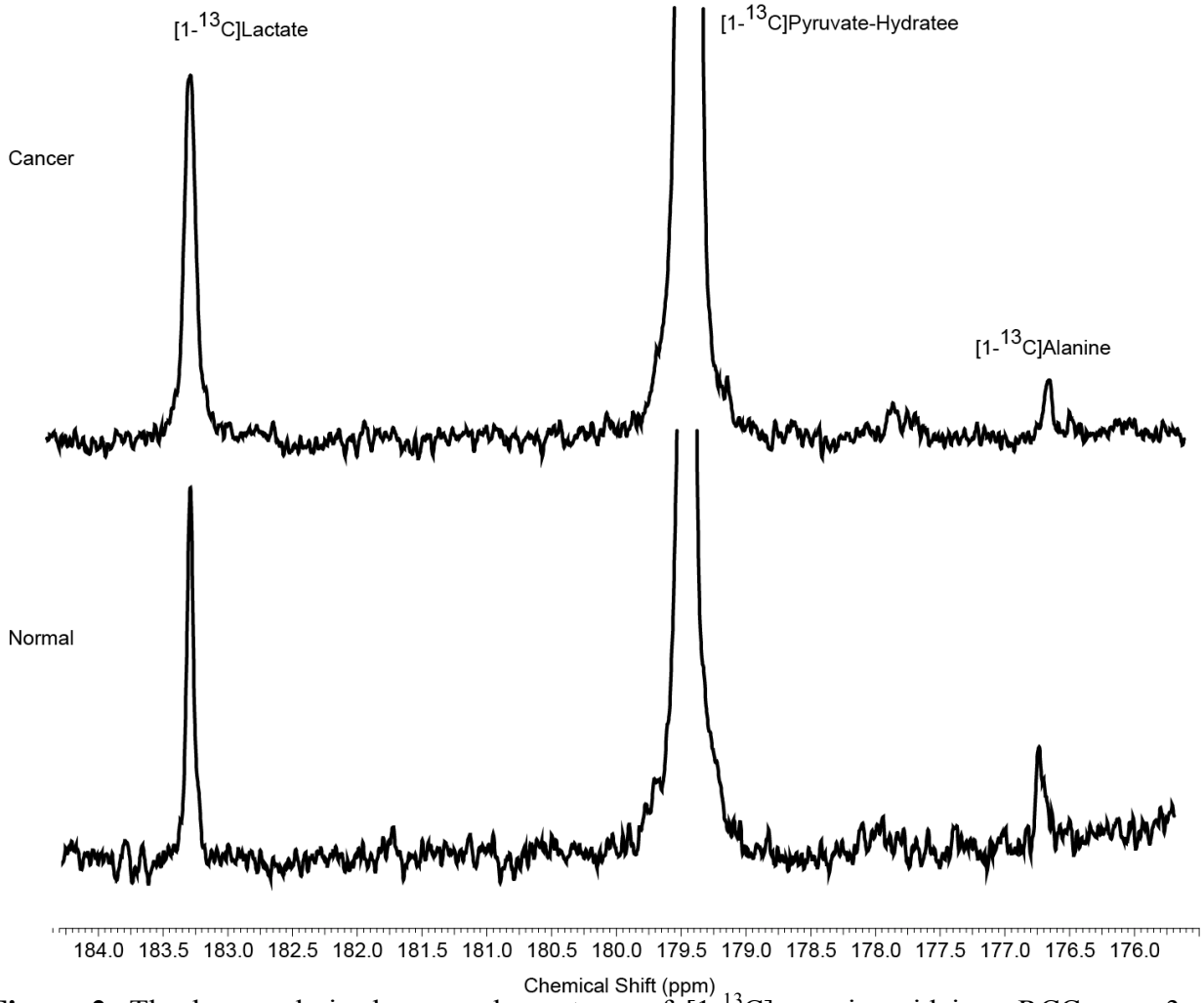


Figure 2. The hyperpolarized summed spectrum of [1-^{13}C]pyruvic acid in ccRCC over 3 minutes, zoomed into the metabolic products region.

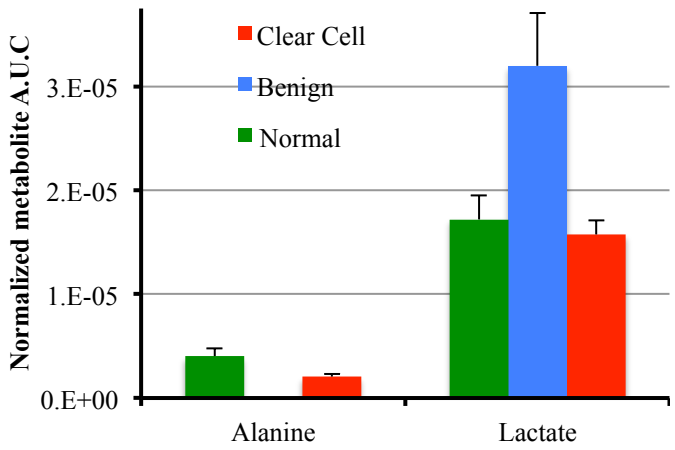
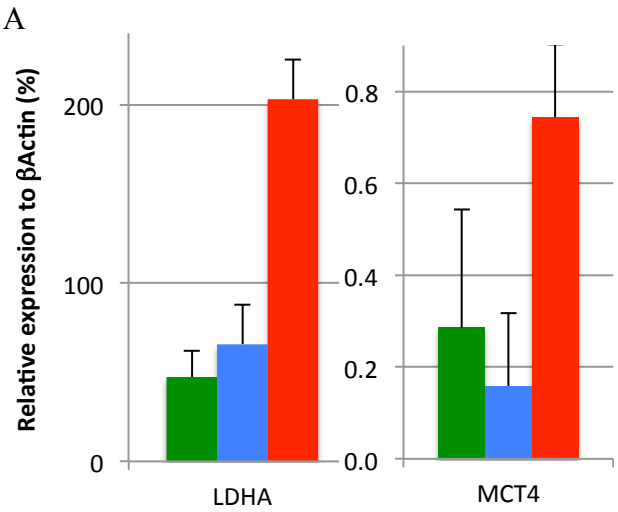


Figure 3. Normalized hyperpolarized metabolite signals measured in various renal tissues.

and lactate signals in normal renal tissues, benign renal tumors, and ccRCCs. Hyperpolarized alanine signal was observed in only 2 out of 9 of the ccRCC cases. In contrast, alanine was more consistently observed in the normal renal tissue slices. This likely reflects a metabolic shift that occurs in ccRCC, with re-routing of pyruvate to lactate, rather than to alanine for protein synthesis. We observed higher hyperpolarized lactate level in benign renal tumors compared to normal renal tissues, and this would be consistent with increased glycolysis and lactate production in the benign tumors compared to normal renal tissues. Paradoxically, the observed hyperpolarized lactate signal in ccRCC, which is expected to be higher than benign renal tumors, was similar to that in normal renal tissues. This likely reflects rapid lactate efflux in ccRCC. In the bioreactor with a continuous flow of medium, extracellular lactate that has flown out of the active region of the MR coil would not be expected to contribute to the observed hyperpolarized lactate signal. The rapid lactate efflux in ccRCC is supported by mRNA expression and immunohistochemical stain of the monocarboxylate transporter 4 (MCT4) and thermal labeling of renal tissues with pyruvate. MCT4 modulates lactate transport from the intracellular compartment to the extracellular compartment. We found significantly higher MCT4 mRNA expression and MCT4 immunochemical staining in ccRCC compared to that in benign renal tumors and normal renal tissue (**figure 4**), consistent with MCT4 modulated increased lactate efflux in ccRCC. We further confirmed increased lactate efflux in ccRCC via thermal labeling of renal tissues with 3-^{13}C pyruvate and measuring lactate in the media. We observed three fold higher rate of lactate secreted into the media by the ccRCC tissue compared to the normal kidney (**figure 5**). This is consistent with the gene expression data for MCT4, which is 2.6 times higher ccRCC compared



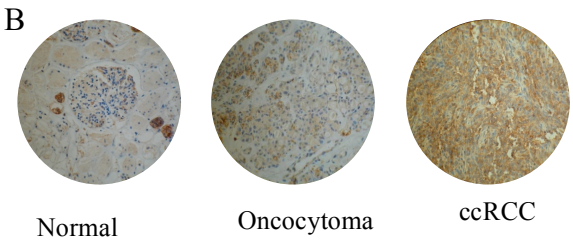


Figure 4*:* Gene expression of LDH-A and MCT4. B)MCT4 staining of renal tissue slices show intense staining in the ccRCC compared to the normal or benign tumors (oncocytoma).

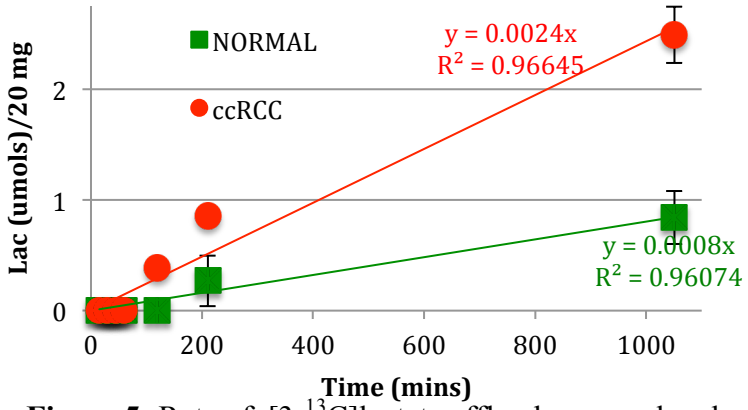


Figure 5*:* Rate of [3-^{13}C]lactate efflux by normal and ccRCC tissue slices , measured using high resolution magnetic resonance spectroscopy.

with normal renal tissues. We also observed significantly higher lactate dehydrogenase-A (LDH-A) mRNA expression in ccRCC compared to benign tumors and normal renal tissues. LDH-A mediates the conversion of pyruvate to lactate. The higher LDH-A expression is consistent with increased glycolysis and lactate production in cancer.

Figure 3 shows the bar graph of the hyperpolarized products of pyruvate metabolized by the tissue slices. The normal renal tissue produced twice as much alanine compared to ccRCC. This likely reflects a metabolic shift in ccRCC with re-routing of pyruvate to lactate, rather than to alanine for protein synthesis. The hyperpolarized lactate levels were significantly high in the benign tumors compared to normal renal tissue ($p=0.014$). However the RCCs demonstrated similar hyperpolarized lactate production as normal renal tissue and can be attributed to the rapid lactate efflux via the upregulated monocarboxylate transporter (MCT4) in ccRCCs compared with normal tissues and benign renal tumors.

Figure 4 shows that the differential expression of lactate dehydrogenase (LDH) enzyme and the monocarboxylate transportes in the different renal tissues (normal (n=4), benign tumors(n=3) and RCC (n=2)). The LDHα enzyme is responsible for the conversion of pyruvate to lactate. As expected, RCC has a significant upregulation of LDHα compared to the normal renal tissue (4 fold, $p<0.005$) as well as benign tumors (3 fold, $p<0.05$). An additional factor that is relevant for the hyperpolarized metabolites measured is the monocarboxylate transporters MCT4, which is predominately responsible for lactate efflux. The gene expression data clearly shows that while the normal tissue and benign tumors have similar MCT4 expression, the RCC has an increased expression resulting in increased lactate efflux. Taken together this data suggests that with elevated uptake of pyruvate and LDHα expression (~2.5 times), the RCC tumors would actually produce higher levels of lactate compared to normal or benign tissue.

Aim 2: Orthotopic model of RCC using patient-derived tumor tissue slices

Summary: We showed feasibility of establishing orthotopic model of RCC using patient-derived tumor tissue slices. We also developed new hyperpolarized carbon-13 imaging sequences with higher spatial resolution as well as sensitivity to generate contrast between the mouse kidney and the tumor grafts. This will allow future in vivo metabolic imaging of the renal tumor grafts.

We showed feasibility of engrafting human renal tumor tissues under the renal capsule of the Rag2-IL2g male mice. Depending on the tissue type, the growth of the grafts ranges from 6-10 months. The viability of the tumor grafts were assessed using contrast enhanced MRI. We also performed immunohistochemical-staining (CD31) on the tumor grafts, which revealed the persistence of human vasculature (**figure 6**). A dynamic carbon-13 sequence was developed using a spectrally and spatially selective pulse [7], [8] for simultaneous detection of multiple metabolic products such as lactate, alanine and bicarbonate using variable flip angles on the metabolites to enhance detection. In order to enhance the contrast of the carbon-13 signal in the tumor grafts compared to the high background signal arising from the mouse kidney, several optimizations were explored, such as diffusion weighting and delayed temporal imaging without much success. The major limiting factor for the carbon-13 imaging study was the limited tumor volume due to protracted growth rate.

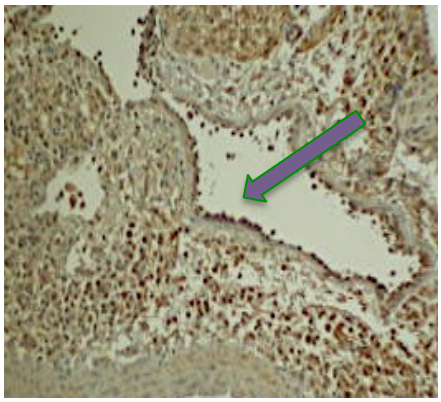


Figure 6: CD31 staining of the graft (arrow) indicates the preserved human vasculature.

We have developed new hyperpolarized carbon-13 imaging sequences with higher spatial resolution as well as sensitivity to generate contrast between the mouse kidney and the tumor grafts. The new sequences developed were based on the balanced steady state free precession (b-ssfp) that Von Morze et al [9] and Reed et al [10]. **Figure 7A** shows the T2-weighted proton image with the red region demarcating the implanted tumor. **Figure 7B** with an in plane resolution of 1mm, clearly demonstrates localization of the lactate signal in the tumor.

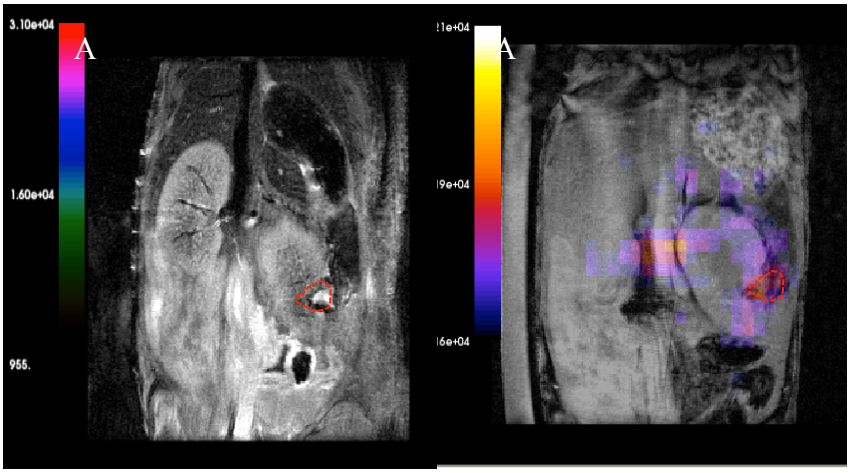


Figure 7: Hyperpolarized Lactate images overlaid on T1 weighted proton reference image. A) T2 weighted proton image of the mouse with the red region outlining the tumor tissue. B)Maximum intensity projection of lactate acquired 15 sec post injection of hyperpolarized pyruvate.

FUTURE DIRECTIONS

a) With the use of either [1,2-$^{13}C_2$]pyruvic acid or [2-^{13}C]pyruvic acid, it will become feasible to capture the tricarboxylic acid (TCA) cycle intermediate metabolites. As TCA cycle is expected to be altered in RCCs, interrogation of TCA metabolites may further help to distinguish between malignant and benign tumors. **Figure 8** shows a representative example of [1,2-^{13}C]pyruvate metabolism in a case urothelial cancer, with

dynamic production of TCA cycle intermediates. In addition to lactate, we were also able to observe formation of bicarbonate and carbon-dioxide (CO_2), indicating visualization of the TCA cycle metabolism. Further optimization with this dual labeled pyruvate is in progress.

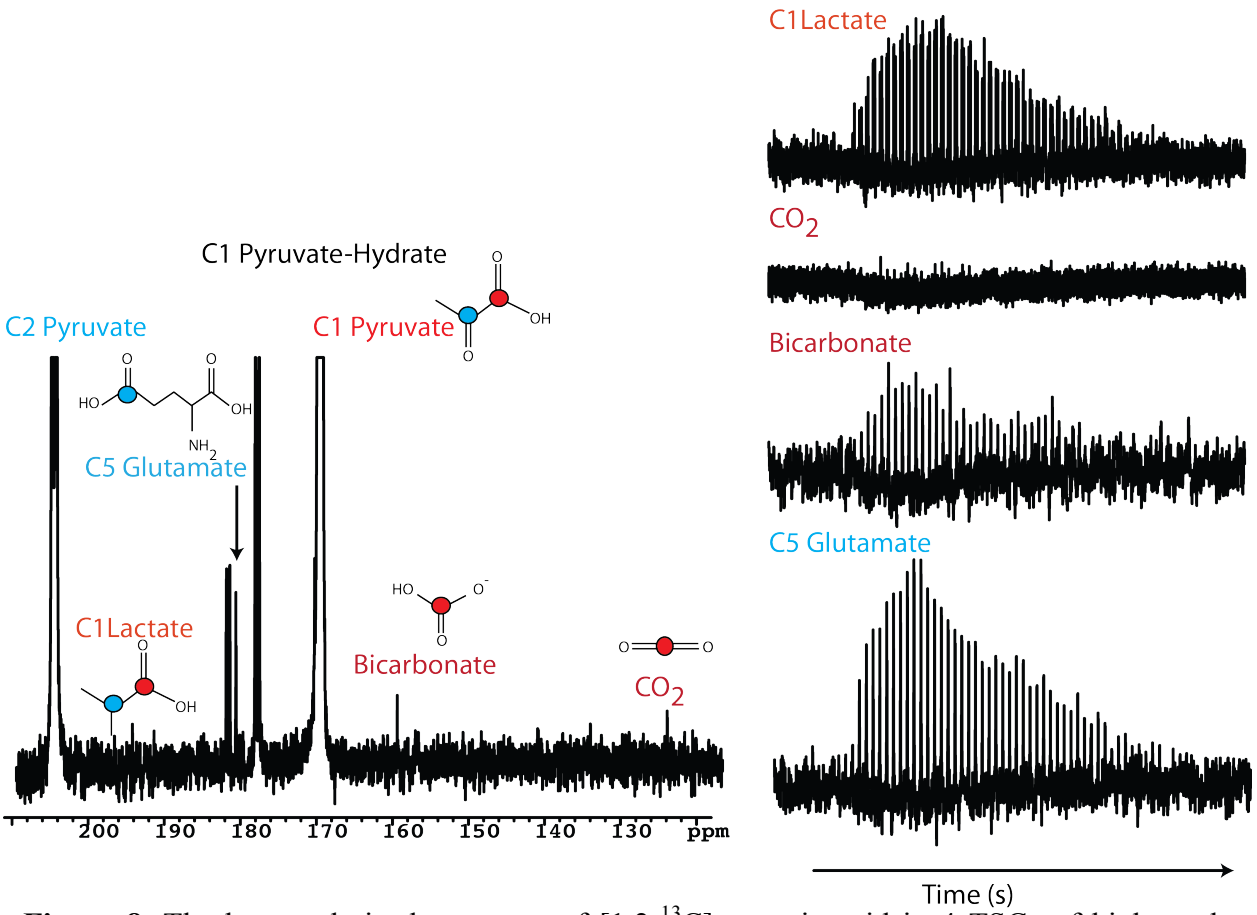


Figure 8. The hyperpolarized spectrum of [1,2-^{13}C] pyruvic acid in 4 TSCs of high grade urothelial cancer. The inset shows the dynamic production of [1-^{13}C]lactate, bicarbonate, carbon-di-oxide and [5-^{13}C]glutamate, indicating TCA activity.

b) To corroborate the lactate efflux that is dominant in the malignant RCCs, further analysis of the media collected from the bioreactor immediately after a hyperpolarized experiment using mass spectrometry to quantify the lactate in the media is underway.Additionally, the tissues labeled with [3-^{13}C]pyruvic acid will be extracted for a comprehensive metabolite examination of the total lactate pools and other intermediates for a complete mass balance analysis.

c) Because of slow growth of benign renal tumors and indolent RCCs in vivo in the orthotopic model, it is challenging to obtain tumor grafts from these indolent tumors that are large enough to allow hyperpolarized MRI. We propose to focus future studies on orthotopic tumor grafts of more aggressive RCCs and further develop in vivo imaging of pyruvate metabolism in RCCs. Collaborators at UCSF have recently developed techniques to acquire hyperpolarized diffusion weighted imaging to provide information

about the localization of the hyperpolarized metabolites *in vivo*. Because lactate efflux appears to be a key feature for RCC, we will apply this new technique in orthotopic tumor grafts to interrogate whether diffusion weighted HP pyruvate MR can provide information on the localization of lactate in RCCs *in vivo*.

KEY RESEARCH ACCOMPLISHMENTS:

- Initial validation of lactate efflux as a characteristic feature of RCC using patient-derived tumor tissues
- Demonstration that hyperpolarized carbon-13 magnetic resonance technique could potentially serve to differentiate between benign tumors and RCCs based on lactate efflux.
- Development of high resolution fast carbon-13 imaging sequences to facilitate measurement of real time metabolism in orthotopically implanted tumors

CONCLUSION:

In the second year, we have shown increased lactate production in renal tumors in general, and importantly, increased lactate efflux in aggressive renal cancers, using patient derived tissue slice cultures. In the last year, we hope to complete the ex vivo bioreactor studies and publish the findings of stratifying renal tumors aggressiveness based on hyperpolarized lactate production and efflux. Subsequently, we hope to implement the *in vivo* model system and use hyperpolarized carbon-13 technique to monitor therapeutic efficacy.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

- Poster Presentation: Renuka Sriram, Kayvan R Keshari, Mark Van Criekinge, John Kurhanewicz, David M Wilson, Donna M Peehl, Robert Bok and Zhen J Wang. "***Patient-Derived Tissue Culture Model Systems of Renal Cell Carcinoma for development of Clinically Translatable Metabolic Biomarkers*"** presented at the American Association for Cancer Research special conference titled '*The Translational Impact of Model Organisms in Cancer*.' 2013, San Diego, USA
- Poster Presentation: Renuka Sriram, Mark Van Criekinge, Ailin Hansen, Kayvan R Keshari, Justin Delos Santos, David M Wilson, Donna M Peehl, John Kurhanewicz and Zhen J Wang. "***Metabolic Dynamics of Patient-derived Renal Cell carcinoma Tissues Using Clinically Translatable Hyperpolarized ^{13}C Pyruvate*"** presented at the World Molecular Imaging Congress (WMIC) 2014, Seoul, Korea.
- Oral Presentation: Renuka Sriram, Mark Van Criekinge, Ailin Hansen, Zhen J Wang, David M Wilson, Kayvan R Keshari and John Kurhanewicz and. "***Real time measurement of hyperpolarized lactate production and efflux as a biomarker of tumor aggressiveness in a MR compatible 3D cell and tissue culture bioreactor*"** to be presented at the ISMRM Workshop on *Magnetic Resonance in Cancer: Challenges & Unmet Needs* in Austin, TX, USA in November 2014.

REPORTABLE OUTCOMES:

- Using patient-derived renal tumor tissues, we show that increased lactate efflux is a characteristic feature that can be used to differentiate benign tumors from RCCs.

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